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CCR5 mediates HIV-1 Tat-induced neuroinflammation and influences morphine tolerance, dependence, and reward

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Abstract

The HIV-1 regulatory protein, trans-activator of transcription (Tat), interacts with opioids to potentiate neuroinflammation and neurodegeneration within the CNS. These effects may involve the C-C chemokine receptor type 5 (CCR5); however, the behavioral contribution of CCR5 on Tat/opioid interactions is not known. Using a transgenic murine model that expresses HIV-1 Tat protein in a GFAP-regulated, doxycycline-inducible manner, we assessed morphine tolerance, dependence, and reward. To assess the influence of CCR5 on these effects, mice were pretreated with oral vehicle or the CCR5 antagonist, maraviroc, prior to morphine administration. We found that HIV-1 Tat expression significantly attenuated the antinociceptive potency of acute morphine (2 – 64 mg/kg, i.p.) in non-tolerant mice. Consistent with this, Tat attenuated withdrawal symptoms among morphine-tolerant mice. Pretreatment with maraviroc blocked the effects of Tat, reinstating morphine potency in non-tolerant mice and restoring withdrawal symptomology in morphine-tolerant mice. Twenty-four hours following morphine administration, HIV-1 Tat significantly potentiated (~3.5-fold) morphine-conditioned place preference and maraviroc further potentiated these effects (~5.7-fold). Maraviroc exerted no measurable behavioral effects on its own. Protein array analyses revealed only minor changes to cytokine profiles when morphine was

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administered acutely or repeatedly; however, 24 h post morphine administration, the expression of several cytokines was greatly increased, including endogenous CCR5 chemokine ligands (CCL3, CCL4, and CCL5), as well as CCL2. Tat further elevated levels of several cytokines and maraviroc pretreatment attenuated these effects. These data demonstrate that CCR5 mediates key aspects of HIV-1 Tat-induced alterations in the antinociceptive potency and rewarding properties of opioids.

Keywords

Conditioned Place Preference; C-C motif chemokines; HIV-associated neurocognitive disorders; Maraviroc; NeuroAIDS; Neuroinflammatiory cytokines; Opiate dependence; Opiate tolerance; Precipitated withdrawal; Warm-Water Tail-Withdrawal Test

1. Introduction

There is a dynamic relationship between opioid use, human immunodeficiency virus-1 (HIV-1) acquisition, and disease progression. Worldwide, injection drug use (IDU) accounts for ~30% of new HIV-1 infections outside of sub-Saharan Africa (WHO, 2016). Within the United States, over 3,500 new infections involved IDU in 2015 (CDC, 2016), a year in which overall drug overdose deaths rose another 11%, the majority (63%) of which involved opioids (Rudd et al., 2016). The convergence of the HIV and opioid epidemics is particularly concerning given evidence that opioid usage increases the progression of HIV-1 to acquired immune deficiency syndrome (AIDS) and promotes neurocognitive impairment in humans and non-human primates (Bokhari et al., 2011; Bell et al., 1996, 2002, 2006; Chuang et al., 2005; Donahoe et al., 1993; Kumar et al., 2004, 2006; Rivera et al., 2013). Moreover, HIV-infected individuals are at risk for the development of neuropathic pain (Malvar et al., 2015) for which prescription opioids remain a common treatment (Kremer et al., 2016; Zilliox, 2017). Pharmacological treatment for opioid abuse includes substitution therapies (e.g. methadone, buprenorphine, buprenorphine/naloxone; Moatti et al., 1988; Roux et al., 2008; Sambamoorthi et al., 2000; Woody et al., 2014), which may exert neurotoxic interactions with HIV-1 proteins (Fitting et al., 2014b). As such, the mechanisms and physiological consequences of HIV/opioid interactions need to be understood in order to improve outcomes for HIV-seropositive patients that are pharmacologically managed for pain and/or addiction.

The biological mechanisms that underlie HIV-1 and opioid interactions in the central nervous system (CNS) likely involve the HIV-1 regulatory protein, trans-activator of transcription (Tat). Tat is critical for efficient HIV replication; however, Tat is soluble and can be secreted from infected cells to exert direct and indirect neurotoxicity *in vitro* (reviewed in King et al., 2006; Nath et al., 2002). Tat promotes neuroinflammation via NF-κling (El-Hage et al., 2008b; Herbein et al., 2010), upregulation of proinflammatory cytokines [particularly the endogenous β chemokine ligands for the C-C “motif” chemokine receptor type 5 (CCR5): C-C chemokine ligand 3 (CCL3, also known as “macrophage inflammatory protein-1α” or MIP-1α), CCL4 (also known as “macrophage inflammatory protein-1β” or MIP-1β), and CCL5 (also known as “regulated on activation normal T-cell expressed and secreted” or RANTES); El-Hage et al., 2005; Hahn et al., 2010], and

subsequent recruitment of neuroimmune cells promoting neuroinflammation. *In vitro,* morphine exacerbates Tat effects to activate microglia (Bokhari et al., 2009; Gupta et al., 2010; Sorrell & Hauser, 2014), increase cytokine production (Bokhari et al., 2009; El-Hage et al., 2005; Fitting et al., 2014b; Turchan-Cholewo et al., 2009), drive oxidative stress (Dalvi et al., 2016; Fitting et al., 2014a,b; Malik et al., 2011; Turchan-Cholewo et al., 2009), increase intracellular calcium (El-Hage et al., 2005; Fitting et al., 2014a,b), and promote neurotoxicity (Fitting et al., 2014a,b; Gurwell et al., 2001; Malik et al., 2011). Morphine and Tat interactions may depend on μ opioid receptors (MORs) given that neurotoxic synergy is observed in co-cultures when mixed glia express MORs, but not when they are derived from $MOR^{-/-}$ mice (Zou et al., 2011). These data support the notion that glial MORs are critical for the indirect neurotoxic effects of Tat.

The proinflammatory effects of HIV-1 Tat at CCR5 may directly influence opioid sensitivity. In studies of opioid-mediated antinociception in rats, activation of CCR5 or CXCR4 can rapidly (within 30 min) desensitize μ- or δ-opioid-receptors (Chen et al., 2007). Blocking actions at CCR5 in proinflammatory states may attenuate heterologous desensitization of MORs and increase therapeutic efficacy. In support, intrathecal administration of the CCR5 antagonist, maraviroc, attenuated chronic constriction injury-induced microgliosis, astrogliosis, upregulation of CCR5 protein, and mRNA expression of CCR5-ligands (CCL3, CCL4, and CCL5) in the spinal cord and dorsal root ganglion concurrent with reduced neuropathic pain (Kwiatkowski et al., 2016). Moreover, CCR5 and MORs may form functionally active heteromers. A bivalent ligand derived from a MOR agonist (oxymorphone) and a CCR5 antagonist (TAK-220) had ~2000× greater antinociceptive potency than morphine in mice experiencing LPS-mediated inflammation (Akgün et al., 2015). Another bivalent ligand comprised of an opioid receptor antagonist (naltrexone) and maraviroc reduced the infectivity of human astrocytes when cultured with R5-tropic HIV (Arnatt et al., 2016; El-Hage et al., 2013; Yuan et al., 2013). These data suggest a dynamic relationship between MOR and CCR5 activation that may contribute to HIV pathology; however, the functional effects are poorly understood. As such, we investigated morphine tolerance, dependence, and reward in a transgenic murine model that conditionally-expresses the proinflammatory HIV-1 regulatory protein, Tat_{1-86}. Using a transgenic mouse approach, conditional Tat expression has been demonstrated to reduce the antinociceptive potency of morphine (Fitting et al., 2012, 2016), while potentiating psychostimulant reward in acute drug withdrawal (24 h post drug administration; Paris et al., 2014a,b). We hypothesized that HIV-1 Tat expression would attenuate morphine antinociceptive potency and that the CCR5 antagonist, maraviroc, would reverse these effects. Further, we hypothesized that 24 h post morphine, Tat- and cytokine-mediated effects would be potentiated.

2. Materials and Methods

The use of mice in these studies was pre-approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University and the experiments were conducted in accordance with ethical guidelines defined by the National Institutes of Health (NIH Publication No. 85-23).

2.1. Subjects and housing

Adult male mice expressed (or did not express) an HIV-1_{IIIB} $tat_{1\text{-}86}$ transgene (N = 245) as previously described (Hauser et al., 2009; Fitting et al., 2013; Bruce-Keller et al., 2008) and were generated in the vivarium at Virginia Commonwealth University (MCV campus). Briefly, HIV-1 $Tat_{1\text{-}86}$ is conditionally expressed in a CNS-targeted manner via a GFAP-driven, Tet-on promoter (activated by consumption of doxycycline-containing chow) in Tat(+) mice. Tat(−) control littermates express the Tet-on transcription factor without the $tat_{1\text{-}86}$ transgene. While Tat can induce astrogliosis in transgenic mice (El-Hage et al., 2008; Hahn et al., 2015; Paris et al., 2015), this has not been observed to impair Tat production (Fitting et al., 2012; Paris et al., 2014b). Additional control experiments were carried out in adult, male, C57BL/6J mice (N = 30). All mice (~70 days of age) were housed 4 - 5 / cage and were maintained in a temperature- and humidity-controlled room on a 12:12 h light / dark cycle (lights off at 18:00 h) with *ad libitum* access to food and water.

2.2. Chemicals

To induce HIV-1 $Tat_{1\text{-}86}$ expression, Tat(+) transgenic mice [and Tat(−) controls] were placed on doxycycline chow (Dox Diet #2018, 6 g/kg; Harlan Laboratories, Madison, WI, USA) for 28 days, unless otherwise specified. Some mice received subcutaneous implants of placebo or morphine pellets (75 mg; National Institute on Drug Abuse, Rockville, MD, USA), the latter of which induces tolerance and dependence in the present strain of mice (Fitting et al., 2016). To precipitate morphine withdrawal, mice were administered (−)naloxone (1 mg/kg, s.c.; Sigma-Aldrich, St. Louis, MO; Fitting et al., 2016). To investigate morphine reward, mice were administered morphine sulfate (#M8777; Sigma-Aldrich) at a concentration of 10 mg/kg, i.p. (0.1 ml per 10 g body weight) which has been demonstrated to produce morphine-conditioned place preference (CPP; Zhu et al., 2015). To investigate the contribution of CCR5 to these effects, some mice were administered the CCR5-selective antagonist, maraviroc (62 mg/kg, p.o.; #376348-65-1; BOC Sciences, Shirley, NY, USA) which was dissolved in 100% DMSO, then diluted to 5% DMSO in vegetable oil. Using inter-species allometric scaling (by a factor of 12.3; Freireich et al., 1966; Reagan-Shaw et al., 2008) others have determined maraviroc (62 mg/kg, p.o.) dosing for mice from clinical formulations (Neff et al., 2010).

2.3. Surgical manipulation

Mice received subcutaneous implants of placebo or morphine (75 mg) pellets under isoflurane (2.5 %) anesthesia as previously reported (Fitting et al., 2016; Ross et al., 2008). Following surgery, mice were monitored to ensure weight gain, muscle tone, proper neurological response, and general health (Crawley and Paylor, 1997). No mice failed to recover.

2.4. Behavioral assays

Prior to all behavioral testing, mice were acclimated to the testing room for 24 h. For assessments of morphine tolerance and morphine dependence, mice received subcutaneous implants of placebo or morphine pellets. Five days later, mice were assessed for morphine tolerance in a warm-water tail-withdrawal assay. Following tail-withdrawal testing, mice

underwent naloxone-precipitated withdrawal and were assessed for morphine dependence. For assessments of morphine reward, Tat-transgenic mice were assessed in a CPP paradigm with psychomotor sensitization assessed on conditioning days. Some mice were additionally assessed on a rotarod to rule out potential locomotor confounds.

2.4.1. Warm-water tail-withdrawal test—A warm-water tail-withdrawal test was conducted with a water-bath maintained at 56 ± 0.1°C as previously described (Coderre & Rollman, 1983; Fitting et al., 2016). Briefly, mice were gently wrapped in a cloth and the distal one-third of the tail was immersed in a water bath. The mice rapidly removed their tail from the bath at the first sign of discomfort and the tail-withdrawal latency was recorded. Tail-withdrawal latency was then assessed using a cumulative dosing procedure. Mice were injected with a starting dose of morphine and were tested for antinociception 20 min later. Mice that did not reach a 10 sec cut-off threshold received an additional cumulative dose of morphine and were retested. This process was repeated until the animals reached the cut-off value of 10 sec. Baseline latency ranged from 2 to 4 sec. The 10 sec maximum cutoff latency was used to prevent tissue damage. Antinociception was quantified as the percentage of maximal possible effect (%MPE): $\%MPE = [(Test\ latency - Baseline\ latency) / (10 - Baseline\ latency)] \times 100$ (Harris & Pierson, 1964).

2.4.2. Antagonist-precipitated withdrawal—Mice were administered an injection of the opioid receptor antagonist, naloxone (1 mg/kg, s.c.), in order to precipitate withdrawal. The primary symptom assessed was jumping from an elevated platform (32 cm high, 17 cm diameter). The proportion of mice that jumped from their individual platforms was recorded over a 10 min trial. The proportion of jumping mice is considered an index of withdrawal (Fitting et al., 2016). This test was followed by an evaluation of additional signs of withdrawal. Mice were placed in a rectangular, clear, plastic observation box (16 × 16 × 30 cm) and observed for 5 min. The concomitant number of jumps, forepaw tremors, and wet-dog shakes was recorded. The frequency of jumps, tremors, and shakes are considered additional indices of withdrawal (Fitting et al., 2016).

2.4.3. Conditioned Place Preference—Behavior in the CPP test was recorded and digitally-encoded by an ANY-maze behavioral tracking system (Stoelting Co., Wood Dale, IL, USA). Morphine-CPP and locomotor sensitization were assessed simultaneously (Zhu et al., 2015). CPP was conducted as modified from previous methods (Paris et al., 2014a). The apparatus (#64101; Stoelting Co.) consisted of two black conditioning chambers (18 × 20 × 35 cm), each visually-distinguished by the presence of white circles or horizontal stripes on the chamber walls, as well as ~30 lux difference in ambient lighting. Conditioning chambers were connected by a start box/transition chamber (10 × 20 cm). A biased conditioning design was utilized (Semenova et al., 1995). On day 1, mice were allowed to freely explore the apparatus for 10 min in order to establish an initial chamber-preference (there was no significant side preference observed across experimental groups). On days 2 - 5 mice underwent one cycle of morphine conditioning per day (receiving an i.p. saline injection paired with confinement to the preferred chamber for 30 min, followed 4 h later by an i.p. morphine, 10 mg/kg, injection paired with confinement to the less preferred chamber for 30 min). Twenty-four hours after the last morphine-conditioning cycle, mice were allowed to

freely explore the apparatus in order to assess their final chamber preference. The amount of time that mice spent in the chambers or the start/transition box, as well as the distance traveled, and the frequency of rearing was recorded on each day. CPP was quantified as a difference score: *CPP d-score = (time spent in the morphine-paired chamber) – (time spent in the saline-paired chamber)* (Paris et al., 2014a).

2.4.4. Rotarod—Locomotor coordination was assessed on an accelerated rotarod as previously described (Paris et al., 2013). Briefly, mice were trained to balance on an immobile rotarod (3 cm in diameter and suspended 44.5 cm high; Columbus Instruments, Columbus, OH, USA) for 30 sec. Mice were then trained to navigate the task across two 30 sec fixed speed trials (10 rpm) and two 180 sec fixed speed trials (10 rpm). Lastly, mice were tested on two accelerated speed trials (180 sec max. latency at 0 - 20 rpm). The mean latency to fall from the rotarod and the maximum RPM achieved across the two accelerated trials were utilized as indices for locomotor performance. Decreased latencies to fall and lower maximal RPM on the accelerated test indicate an impaired motor phenotype.

2.5. Cytokine assay

The head of the caudate nucleus has been identified as a central reservoir for maximal HIV viral load in humans (reviewed in Nath, 2015) and we have found the striatum to host a cell population that may be selectively vulnerable to HIV-1 Tat (Schier et al., 2017). As such, cytokine analyses were conducted on caudate/putamen (dorsal striatum) in the present mouse model. Mice underwent cervical dislocation and bilateral dorsal striata were immediately dissected, flash-frozen in liquid nitrogen, and stored at -80°C until assay. At the time of assay, tissues were homogenized in IP lysis buffer (#87787; Pierce Biotechnology, Rockford, IL, USA) with a protease/phosphatase inhibitor cocktail (#04693159001; Roche, Mannheim, Germany). Protein concentrations were determined via bicinchoninic acid (BCA) assay per kit manufacturer instructions (#23224; Pierce Biotechnology).

Cytokines were assessed using a Bio-Plex Pro™ Mouse Cytokine 23-plex assay kit (#M60009RDPD; Bio-Rad Laboratories, Inc., Hercules, CA, USA) and analyzed on a Bio-Plex 200 system. Samples were diluted to a concentration of 500 μg/mL. Unknown samples and standards were incubated with fluorescent, antibody-tagged microspheres and detected via streptavidin-phycoerythrin-labeled detection antibodies. Cytokine concentrations were calculated from respective standard curves via Bio-Plex Manager 4.0 software. All samples were analyzed in duplicate. Limits of detection ranged from 2 to 20 pg/mL. Mean intra- and inter-assay coefficients of variance were 7.2 % and 5.3 %, respectively.

2.6. Statistical analyses

Median effective doses (ED_{50}; reported with 95 % confidence intervals) were determined via non-linear regression (sigmoidal curvilinear modeling with variable slope) using a least-squares fit for each treatment group (bottom and top values constrained to 0 and 100, respectively). Dependent measures for additional behavioral analyses were assessed via ANOVA (to assess dependence) or repeated measures ANOVA (to assess psychostimulation or CPP over multiple trials) with drug condition (placebo or morphine), inhibitor condition (vehicle or maraviroc), and genotype [Tat(−) or Tat(+)] as factors. Cytokine arrays were

assessed via separate three-way ANOVAs. Mice treated acutely with doxycycline (48 h) were analyzed with morphine condition (acute saline or acute morphine), inhibitor condition (vehicle or maraviroc), and genotype [Tat(−) or Tat(+)] as factors. Mice treated chronically with doxycycline for 28 days were analyzed with morphine condition (morphine-naïve, repeated morphine, or 24 h post morphine), inhibitor condition (vehicle or maraviroc), and genotype [Tat(−) or Tat(+)] as factors. Fisher's Protected Least Significant Difference *post-hoc* tests determined group differences following main effects. Interactions were delineated via simple main effects and main effect contrasts with alpha controlled for multiple comparisons. Analyses were considered significant when $p \leq 0.05$.

3. Results

3.1. HIV-1 Tat decreased morphine potency in non-tolerant mice; tolerance or pharmacological antagonism of CCR5 attenuated Tat effects

As negative control measures before proceeding to tests involving Tat-tg mice, morphine dosing, non-specific interactions with maraviroc, and warm-water tail-withdrawal test conditions were confirmed in C57BL/6J mice. In a 52°C water bath, morphine administered at 5 mg/kg (Fig. 1A) or 10 mg/kg (Fig. 1B) produced antinociception that was present for at least 2 h and peaked at 60 min, commensurate with observations in other animal models (Altun et al., 2015; Williams et al., 2008). In a 56°C water bath, cumulative morphine-dosing produced antinociception commensurate with what we have previously observed in C57BL/6J mice (Fitting et al., 2016; Fig. 1C). Maraviroc did not significantly influence the acute time-course (Fig. 1A-B) or the antinociceptive effects of morphine in C57BL/6J mice [vehicle/morphine ED_{50} = 2.0 (95 % CI: 0.9 – 4.3), maraviroc/morphine ED_{50} = 3.0 (95% CI: 2.2 – 4.1)] (Fig. 1C).

Tat(−) and Tat(+) mice were placed on doxycycline for 28 days to induce Tat expression (or not). On day 28, mice were implanted with a subcutaneous placebo pellet or morphine pellet to induce morphine tolerance. From days 28 – 32, mice were administered vehicle or maraviroc (p.o., QD). On day 33, mice received their last dose of vehicle or maraviroc and were tested 30 min later.

Tat induction, morphine tolerance, and maraviroc pretreatment significantly influenced the antinociceptive response to acute morphine (Fig. 2A-B). Among non-tolerant, placebo-pelleted mice, Tat exposure produced a modest but significant shift to the right in the antinociceptive potency of acutely-administered morphine [$F(1,48) = 7.97$, $p < 0.05$] [Fig. 2A; $Tat(-)_{ED50}$ = 4.5 (95% CI: 3.6 - 5.5), $Tat(+)_{ED50}$ = 6.8 (95% CI: 5.6 - 8.0)]. This effect was obviated by maraviroc pretreatment [Fig. 2B; $Tat(-)_{ED50}$ = 6.0 (95% CI: 4.9 - 7.1), $Tat(+)_{ED50}$ = 5.8 (95% CI: 4.9 - 6.7)]. Morphine tolerance significantly shifted the ED_{50} for morphine-induced antinociception to the right in all treatment groups [Fig. 2A-B; ED_{50} range = 17.8 - 53.8]. Notably, baseline tail-withdrawal latencies were ~0.4 sec greater among morphine-pelleted (2.7 ± 0.1 sec) vs. placebo pelleted mice (2.3 ± 0.1 sec) [$F(1,44) = 10.91$, $p < 0.05$]. Neither Tat induction nor maraviroc pretreatment influenced baseline tail-withdrawal latencies.

3.2. Among tolerant mice, Tat-exposure decreased primary withdrawal behavior; pharmacological antagonism of CCR5 attenuated Tat effects

Following testing for antinociception, mice were administered the opioid receptor antagonist, naloxone, and precipitated withdrawal behaviors were assessed. Tat induction, morphine tolerance, and maraviroc pretreatment significantly interacted to influence the primary measure of withdrawal, the proportion of mice that jumped from an elevated platform [$F(1,44) = 6.77$, $p < 0.05$] (Fig. 3A). Unlike other withdrawal-related behaviors assessed, elevated platform jumping was only observed among previously morphine-tolerant mice (a significant difference from their placebo-pelleted counterparts [$F(1,44) = 122.27$, $p < 0.05$]; Fig. 3A). Among morphine-tolerant mice, withdrawal- precipitated jumping was significantly attenuated by Tat exposure [Tat(+) mice significantly differed from Tat(−) controls ($p = 0.03$)] or maraviroc pretreatment [maraviroc-treated, Tat(−) mice significantly differed from Tat(−) controls ($p = 0.005$)] (Fig. 3A). However, combined Tat exposure and maraviroc pretreatment restored withdrawal-precipitated jumping [maraviroc-treated Tat(+) mice significantly differed from maraviroc-treated Tat(−) mice ($p = 0.004$) or vehicle-treated Tat(+) mice ($p = 0.03$)] (Fig. 3A).

When observed for additional, simultaneous withdrawal-precipitated behaviors the frequency of spontaneous jumping was significantly greater [$F(1,44) = 10.83$, $p < 0.05$] (Fig. 3B) and paw tremors were significantly reduced [$F(1,44) = 10.79$, $p < 0.05$] (Fig. 3C) among morphine-tolerant mice compared to placebo-pelleted controls irrespective of Tat or maraviroc exposure. There was a main effect for wet-dog shakes to be significantly greater among Tat(+) mice compared to Tat(−) mice, irrespective of morphine or maraviroc exposure [$F(1,44) = 3.97$, $p = 0.05$] (Fig. 3D).

3.3. HIV-1 Tat exposure and CCR5 antagonism attenuated the psychomotor response to morphine

Both Tat exposure and maraviroc pretreatment interacted with repeated morphine injections to influence locomotor behavior (Fig. 4AB). Among vehicle-pretreated mice, morphine administration significantly increased the distance traveled in the CPP apparatus [$F(7,147) = 16.66$, $p < 0.05$] compared to saline administration, irrespective of Tat exposure ($p < 0.0001$ per day; Fig. 4A). However, among maraviroc-pretreated mice, Tat-exposure and morphine administration significantly interacted [$F(7,147) = 2.46$, $p < 0.05$] such that Tat(−) mice receiving morphine demonstrated significantly greater distances traveled on days 1 ($p = 0.03$) and 4 ($p = 0.003$), compared to Tat(+) mice receiving morphine (Fig. 4B). Tat(−) mice also displayed enhanced locomotion to a significantly greater degree following morphine-administration than following saline-administration ($p < 0.0001$ per day); whereas, locomotion among Tat(+) mice did not differ between morphine or saline administration (Fig. 4B). Notably, Tat exposure can impair locomotion on its own; however, these effects are not usually observed in the present model until Tat has been induced for over one month (Hahn et al., 2012). To rule out non-specific effects of Tat on locomotion, some Tat(−) and Tat(+) mice from the present experiment were assessed on an accelerated rotarod after testing. No significant differences in locomotor capacity were observed following fixed speed trials (10 rpm) lasting 30 s [Tat(−): 9 ± 2, Tat(+): 12 ± 2] or 180 s [Tat(−): 56 ± 16,

Tat(+): 81 ± 20], or following accelerated speed trials (0 – 20 rpm) lasting 180 s [Tat(−): 132 ± 10, Tat(+): 128 ± 14].

3.4. CCR5 antagonism potentiated Tat-related morphine reward

To assess the contribution of HIV-1 Tat on the rewarding properties of morphine, mice were assessed for morphine-CPP. Tat(−) and Tat(+) mice were exposed to two morphine conditioning cycles (saline followed by morphine) and assessed for chamber preference 24 h after the last morphine administration (Fig. 5A). Following two conditioning cycles, morphine significantly increased the time that mice spent in the previously non-preferred chamber [$F(1,24) = 26.84$, $p < 0.05$] (Fig. 5A′). However, significant differences between Tat(−) and Tat(+) mice were not observed (Fig. 5A′).

Another group of mice underwent four morphine-conditioning cycles and were assessed for final chamber preference 24 h later (Fig. 5B). Under these conditions, Tat genotype significantly interacted with the final preference trial [$F(1,22) = 5.43$, $p < 0.05$] (Fig. 5B′). Tat(−) and Tat(+) mice spent a commensurate amount of time in the least-preferred chamber prior to conditioning; but, Tat(+) mice spent a significantly greater amount of time in the morphine-paired chamber following four cycles of morphine conditioning compared to Tat(−) controls ($p = 0.04$; Fig. 5B′).

To assess the influence of CCR5 on Tat-potentiated CPP, Tat(−) and Tat(+) mice were pretreated with vehicle or maraviroc 30 min prior to the start of conditioning over four cycles (Fig. 5C). As previously observed, mice significantly preferred the morphine-paired chamber 24 h following conditioning, irrespective of treatment [$F(1,42) = 100.80$, $p < 0.05$] (Fig. 5C′). There was significant interaction between Tat-genotype and maraviroc condition [$F(1,42) = 4.13$, $p < 0.05$], such that Tat(+) mice spent a significantly greater amount of time in the morphine-paired chamber when pretreated with maraviroc than did any other group ($p = 0.0006 – 0.02$; Fig. 5C′).

3.5. Prior morphine exposure and HIV-1 Tat increase striatal cytokine content and CCR5 antagonism attenuates Tat effects

Cytokine protein expression was assessed in the dorsal striatum (caudate/putamen) of Tat(−) and Tat(+) mice. In order to parse the acute- vs. chronic influences of Tat, maraviroc, and morphine on the immune effectors examined, tissues from two separate groups of mice were assessed. The first group was acutely exposed to Tat (48 h of doxycycline to induce Tat), maraviroc (a single administration of vehicle or maraviroc, 62 mg/kg, p.o., 30 min prior to i.p. injection), and morphine (a single administration of saline or morphine, 10 mg/kg, i.p.). The second group was chronically exposed to Tat (28 d of doxycycline to induce Tat), repeated maraviroc (vehicle or maraviroc, 62 mg/kg, p.o., QD for 4 d, 30 min prior to i.p. injection), and repeated morphine (saline or morphine, 10 mg/kg, i.p., QD for 4 d). Tissues were collected either 1 h after the last treatment, or 24 h after the last treatment (the latter timeframe being commensurate with that which produced Tat-potentiated morphine-CPP).

3.5.1. Chemotactic Cytokines—Among mice that were acutely-exposed to Tat and drug manipulations (Fig. 6, left-hand panels, 48 h doxycycline exposure), acute Tat induction and

acute maraviroc pretreatment significantly interacted to increase striatal CCL3, compared to vehicle- treated, Tat(−) controls [$F(1,31) = 4.69$, $p < 0.05$] (Fig. 6B, left panel, white bars). There was an additional interaction for acute morphine to significantly increase CCL5, but only among Tat(+) mice [$F(1,31) = 4.59$, $p < 0.05$] (Fig. 6G, left panel, cyan bars). Main effects were observed for acute Tat exposure to significantly increase CCL4 [$F(1,31) = 15.56$, $p < 0.05$] (Fig. 6C, left panel, white and cyan bars) and CCL11 [$F(1,31) = 5.56$, $p < 0.05$] (Fig. 6H, left panel, white and cyan bars), irrespective of maraviroc or morphine administration. Lastly, there was a main effect for morphine to significantly elevate CXCL1 [$F(1,31) = 10.55$, $p < 0.05$], irrespective of Tat or maraviroc exposure (Fig. 6D,left panel, cyan bars). Acute treatments did not otherwise influence the chemokines examined.

Among mice exposed to chronic Tat and repeated morphine, no significant differences were observed until morphine was withheld for 24 h (Fig. 6, right-hand panels, 28 d doxycycline exposure). Three-way interactions were revealed 24 h post morphine treatment for CCL2 [$F(2,45) = 3.64$, $p < 0.05$] (Fig. 6A, right panel, gray bars), CCL3 [$F(2,45) = 6.14$, $p < 0.05$] (Fig. 6B, right panel, gray bars), CCL4 [$F(2,45) = 4.55$, $p < 0.05$] (Fig. 6C, right panel, gray bars), CXCL1 [$F(2,45) = 4.21$, $p < 0.05$] (Fig. 6D, right panel, gray bars), G-CSF [$F(2,45) = 5.42$, $p < 0.05$] (Fig. 6E, right panel, gray bars), and GM-CSF [$F(2,45) = 6.11$, $p < 0.05$] (Fig. 6F, right panel, gray bars). In each case, chronic Tat significantly potentiated the increase of chemokines and maraviroc pretreatment significantly ameliorated this potentiation (see outlined chemokines in Fig. 6). Additionally, a main effect was observed for CCL5 to be increased 24 h post morphine administration, irrespective of Tat or maraviroc exposure [$F(2,45) = 115.48$, $p < 0.05$] (Fig. 6G, right panel, gray bars). Intriguingly, repeated morphine significantly reduced CCL11 [$F(2,45) = 3.25$, $p < 0.05$], an effect that was prevented by maraviroc pretreatment (Fig. 6H, right panel, magenta bars). Once morphine was withheld for 24 h, CCL11 declined in all groups (Fig. 6H, right panel, gray bars).

3.5.2. Pro-inflammatory cytokines—Acute HIV-1 Tat and maraviroc/morphine exposure (Fig. 7, left-hand panels, 48 h doxycycline exposure) influenced pro-inflammatory cytokine levels. A significant interaction was observed for acute morphine to modestly elevate IL-1α, but only in maraviroc-pretreated mice [$F(1,31) = 6.11$, $p < 0.05$] (Fig. 7G, left panel, cyan bars). Acute Tat also significantly increased IL-3 (which was attenuated by maraviroc) [$F(1,31) = 3.82$, $p = 0.05$] (Fig. 7A, left panel, white bars) and IL-9 (which was attenuated by morphine and reinstated by maraviroc) [$F(1,31) = 3.96$, $p = 0.05$] (Fig. 7C, left panel, white and cyan bars). Lastly, there was a main effect for acute Tat exposure to cause a modest, significant increase in IL-1α [$F(1,31) = 7.03$, $p < 0.05$] (Fig. 7G, left panel, white and cyan bars) and a main effect for acute maraviroc to increase IL-6 [$F(1,31) = 4.07$, $p < 0.05$] (Fig. 7B, left panel, white bars). No additional effects of acute treatments were observed on pro-inflammatory cytokines.

In mice exposed to chronic Tat and repeated maraviroc/morphine treatment, significant elevations were observed in all pro-inflammatory cytokines examined (Fig. 7, right-hand panels, 28 d doxycycline exposure). Three-way interactions were revealed 24 h post morphine treatment for IL-3 [$F(2,45) = 6.73$, $p < 0.05$] (Fig. 7A, right panel, gray bars), IL-6 [$F(2,45) = 3.14$, $p = 0.05$] (Fig. 7B, right panel, gray bars), IL-9 [$F(2,45) = 5.18$, $p < 0.05$]

(Fig. 7C, right panel, gray bars), IL-12p40 [$F(2,45) = 4.02$, $p < 0.05$] (Fig. 7D, right panel, gray bars), IL-12p70 [$F(2,45) = 5.43$, $p < 0.05$] (Fig. 7E, right panel, gray bars), and IL-17A [$F(2,45) = 5.65$, $p < 0.05$] (Fig. 7F, right panel, gray bars). In each case, chronic Tat significantly potentiated the increase of proinflammatory cytokines and maraviroc pretreatment significantly ameliorated this potentiation (see outlined cytokines in Fig. 7). A 3-way interaction was also revealed for IFN-γ to be significantly increased among mice that were exposed to chronic Tat and repeated maraviroc/morphine [$F(2,45) = 3.23$, $p < 0.05$] (Fig, 7J, right panel, magenta bars). These effects were significantly exacerbated 24 h post morphine; however, this potentiation was not significantly attenuated by maraviroc-pretreatment (Fig, 7J, right panel, gray bars). Interestingly, repeated morphine also significantly increased IL-9 content in maraviroc-pretreated mice [$F(2,45) = 5.63$, $p < 0.05$] (Fig. 7C, right panel, magenta bars). Irrespective of Tat and maraviroc exposure, main effects were observed for cytokines to be increased 24 h post morphine for IL-1α [$F(2,45) = 80.55$, $p < 0.05$] (Fig. 7G, right panel, gray bars), IL-1β [$F(2,45) = 288.09$, $p < 0.05$] (Fig. 7H, right panel, gray bars), IL-2 [$F(2,45) = 475.92$, $p < 0.05$] (Fig. 7I, right panel, gray bars), and TNF-α [$F(2,45) = 42.36$, $p < 0.05$] (Fig. 7K, right panel, gray bars).

3.5.3. Anti-inflammatory cytokines—Among the anti-inflammatory cytokines assessed, only IL-10 demonstrated a significant response to acute manipulations (Fig. 8, left-hand panels, 48 h doxycycline exposure), [$F(1,31) = 5.56$, $p < 0.05$]. Acute Tat exposure significantly increased IL-10, and this effect was attenuated by maraviroc unless co-administered with acute morphine (Fig. 8A, left panel, white and cyan bars).

When assessed for chronic Tat exposure and repeated maraviroc/morphine administration (Fig. 8, right-hand panels, 28 d doxycycline exposure), IL-10 was the only anti-inflammatory cytokine that demonstrated Tat-mediated potentiation 24 h post morphine that was reversible by pretreatment with maraviroc [$F(2,45) = 6.46$, $p < 0.05$] (Fig. 8A, right panel, gray bars). Other anti-inflammatory cytokines demonstrated significant main effects for enhanced protein expression 24 h post morphine, but not in a Tat- or maraviroc-sensitive manner: IL-4 [$F(1,45) = 499.77$, $p < 0.05$] (Fig. 8B, right panel, gray bars), IL-5 [$F(2,45) = 3.45$, $p < 0.05$]] (Fig. 8C, right panel, gray bars), and IL-13 [$F(2,45) = 313.62$, $p < 0.05$]] (Fig. 8D, right panel, gray bars). Additionally, a main effect for 28 d Tat exposure to significantly increase IL-4 was observed [$F(1,45) = 9.13$, $p < 0.05$], independent of maraviroc or morphine administration (Fig. 8B, right panel, yellow/magenta/gray bars).

4. Discussion

The overall hypotheses that HIV-1 Tat expression attenuates morphine potency, CCR5 antagonism reverses these effects, and withholding morphine for 24 h exacerbates Tat's effects on cytokines were upheld. The present data revealed even greater nuance in the behavioral response to morphine regimens than was anticipated. Consistent with prior reports (Fitting et al., 2012, 2016), HIV-1 Tat expression significantly attenuated the antinociceptive potency of acute morphine injection (2 – 64 mg/kg, i.p.) and significantly attenuated aspects of withdrawal among mice made tolerant via subcutaneous implant of a morphine pellet (75 mg). The present work further extends these findings to demonstrate that pretreatment with the CCR5 antagonist, maraviroc, blocks the effects of Tat on

morphine tolerance and dependence behaviors (reinstating morphine potency in non-tolerant mice and restoring aspects of withdrawal symptomology in morphine-tolerant mice). We further assessed the potential interactions of HIV-1 Tat and maraviroc on morphine reward via a CPP assay. Tat reduced the locomotor response associated with repeated, once-daily, morphine injections (10 mg/kg. i.p.) over 4 days of conditioning, but significantly potentiated (~3.5-fold) morphine-CPP after morphine was withheld for 24 h. These results are congruent with prior findings of Tat-potentiated psychostimulant and ethanol reward (McLaughlin et al., 2014; Mediouni et al., 2015; Paris et al., 2014a,b) and a slower rate of extinction in HIV-1 transgenic rats contextually conditioned to morphine (Homji et al., 2012). Surprisingly, maraviroc further potentiated Tat's effects on morphine-mediated locomotion and CPP (~5.7-fold), but exerted no influence on these behaviors when administered alone. Protein arrays revealed that withholding morphine for 24 h (commensurate with the timeframe of CPP assessment) markedly increased the levels of multiple categories of cytokines (proinflammatory, anti-inflammatory, and regulatory), suggesting a widespread dysregulation of immune function. Importantly, maraviroc could block or attenuate the immunological consequences of terminating morphine administration across a number of cytokines. Tat-dependent elevations in the endogenous CCR5 ligands (CCL3 and CCL4) and CCL2 (whose activation is proposed to be downstream of CCR5-dependent Tat/opioid interactions; El-Hage et al., 2008), as well as other cytokines increased by Tat, were markedly attenuated by maraviroc.

The marked increase in a large number of cytokines that was observed 24 h after withholding repeated morphine (10 mg/kg/d) was unanticipated and may be contributed to by several sources. Although immune function is generally suppressed by sustained opioid exposure (Bayer et al., 1994; Rahim et al., 2003), it can transiently rebound followed by sustained immunosuppression after opioid withdrawal (Rahim et al 2004; Eisenstein et al., 2006). Contextual cues alone (such as those used in CPP) alter immune responding once opiate conditioning is established (Hutson et al., 2017; Saurer et al., 2011), effects that involve the ventral striatum (Szczytkowski et al., 2011). Alternatively, chronic doxycycline treatment can produce alterations in the gut microbiome (Angelakis et al., 2014) which may influence immune responding and subsequent behavior. Interestingly, in this study we found that chronic doxycycline treatment upregulated some but not all chemokines and cytokines in dorsal striatum of both Tat(−) and Tat(+) mice (CCL5, IL-1α, IL-1β, IL-2, IL-6, and IL-13 were upregulated in chronic, compared to acute, doxycycline exposure). There is increasing evidence that microbial dysbiosis can influence many centrally mediated effects, including anxiety and mood (Cryan and Dinan, 2012; Foster et al., 2013). We have previously reported that HIV-1 Tat can induce bacterial translocation and enhance pro-inflammatory cytokines via TLR4 activation in the mouse colon (Guedia et al., 2016). Chronic morphine has also been reported to induce microbial dysbiosis and increase pro-inflammatory cytokines within the gut (Kang et al., 2017; Meng et al., 2013). In particular, morphine pellets have been observed to induce sepsis (Eisenstein et al., 2006; Feng et al., 2005; Hillburger et al., 1997) which may be due, in part, to intestinal stasis. Given that HIV-1 Tat has been shown to increase the permeability of the blood-brain barrier (Leibrand et al., 2017), the presence of Tat may compound the central accumulation of systemic factors including cytokines. The present findings support previous literature demonstrating MOR

and CCR5 interactions on HIV-1 Tat-mediated cellular outcomes and begin to reveal the related behavioral sequelae.

The comorbidity between opioid abuse and HIV is well established. The cellular/molecular interactions between opioids and HIV co-receptors are less well understood, but may exert important effects on chemokine function. Opioids modulate CCR5 expression and signaling *in vitro.* Morphine or methadone upregulates CCR5 mRNA and protein expression in murine BV-2 derived microglia (Bokhari et al., 2009), human astrocytes (Mahajan et al., 2005b), human lymphoid cell lines (Miyagi et al., 2000; Suzuki et al., 2002a), or human monocyte-derived macrophages (Guo et al., 2002; Li et al., 2003) or microglia (Li et al., 2002). These effects are functional and confer susceptibility to simian immunodeficiency virus (SIV; Miyagi et al., 2000; Suzuki et al., 2002a,b) or R5-tropic HIV (Guo et al., 2002; Li et al., 2003). Importantly, CCR5 upregulation and viremia has been shown to be attenuated with opioid receptor antagonists, such as naltrexone (Guo et al., 2002), naloxone (Mahajan et al., 2005b), or the quaternary opioid antagonist, methylnaltrexone (Ho et al., 2003). These effects are, at least partly, dependent on actions at MORs given that the highly selective MOR agonist, DAMGO, upregulates CCR5 and enhances R5-HIV viremia *in vitro*, an effect that is blocked by the MOR-selective antagonist, CTAP (Steele et al., 2003). In addition to altered CCR5 expression, MOR and CCR5 undergo heterologous cross-desensitization (Szabo et al., 2002, 2003; Zhang et al., 2004), which may occur through convergent signaling or via direct molecular interactions involving the formation of MOR-CCR5 heteromers. Evidence for direct MOR-CCR5 dimeric/oligomeric interactions are partly based on findings that a bivalent ligand comprised naltrexone and maraviroc can reduce the infectivity of cultured human astrocytes (El-Hage et al., 2013; Yuan et al., 2013) and attenuate R5 HIV-mediated increases in CCL5, TNF-α, and IL-6 in human astrocytes (El-Hage et al., 2013). Divergent effects of the bivalent ligand were observed in cultured human microglia, which were much more dynamic in their response to morphine. Unlike astrocytes, microglia demonstrated a strong upregulation of CCR5 in response to the bivalent ligand in culture that may have accounted for the differential response between cell types (El-Hage et al., 2013). In other studies, treating a human lymphoid cell line with morphine and CCL4 (respective agonists for MOR and CCR5) increased morphine-mediated upregulation of CCR5 protein expression (Suzuki et al., 2002c). Together, our data agree with prior findings (Szabo et al., 2002, 2003; Zhang et al., 2004) supporting the notion that opioids interact with CCR5 at multiple levels, from the regulation of CCR5 expression and signaling to potential actions at MOR-CCR5 heteromers and functional effects on viremia and neuroinflammation. These present findings begin to extend these data to behavioral dysfunction in a whole-animal model.

Actions at CCR5 may represent a critical point of convergence between the viral protein, Tat, and MORs. Apart from its role in HIV entry, CCR5 signaling is essential in macrophage and microglial chemoattraction, the homing of $CD8^+$ T cells, and the pathogenesis of CNS inflammatory diseases (Martin-Blondel et al., 2016). Foremost, Tat/opioid interactions for neurotoxicity appear to be dependent on glial MORs. While Tat demonstrates neurotoxicity in co-cultured neurons from either wildtype mice or MOR-knockout mice, these effects are potentiated by pretreatment with morphine only when neurons are co-cultured with wildtype glia (MOR-knockout glia do not potentiate toxicity; Zou et al., 2011). Second, Tat/opioid

toxicity largely involves downstream neuroinflammation. In murine progenitors, Tat and opioids have interactive effects on astroglial chemokine secretion (Hahn et al., 2010), CCL5 is often the predominant central chemokine upregulated in response to Tat, an effect that can be potentiated by chronic morphine (Dutta & Roy, 2015), and inactivating CCR5 with an antibody blocks the chemoattractive effects promoted by Tat (Hahn et al., 2010). Third, CCL5 activation appears to work in concert with downstream chemokine activation, particularly CCL2. In support, morphine stimulates production of CCL5 and CCL2 in cultured peripheral blood mononuclear cells (Wetzel et al., 2000) and Tat increases CCL2 and CCL5 production in murine primary astrocyte cultures; effects that are potentiated by co-application of morphine and reversed by MOR antagonists (such as β-FNA; El-Hage et al., 2005). Striatal infusion of Tat_{1-86} to mice significantly increases the proportion of CCL5 immunoreactive astroglia and co-administration of morphine and Tat significantly increases astrogliosis, microgliosis, the proportion of CCL2 immunofluorescent astrocytes/macrophages/microglia, and reactive nitrosative species co-localized to these cells (El-Hage et al., 2008a). These effects on murine striatum are obviated by CCL5 knockout (El-Hage et al., 2008a) or knockout of the CCL2 receptor (El-Hage et al., 2006). Findings in primary human astrocytes are supportive with morphine downregulating CCL2 and CCL4 mRNA and upregulating their receptors in a naloxone-dependent manner (Mahajan et al., 2005b). Thus, Tat and opioids acting at MORs appear to activate CCR5 and CCR2 signaling, increasing neuroinflammation and striatal neurotoxicity. These effects may contribute to the attenuated therapeutic efficacy and increased rewarding properties of morphine that we have observed herein.

We propose that MOR-CCR5 cross-desensitization may contribute to the behavioral pathology observed (Fig. 9). When HIV-1 Tat-mediated neuroinflammation was not present [as modeled using Tat(−) control mice], cytokine concentrations were at basal levels and morphine exerted efficacious effects for tolerance, dependence, psychostimulation, and reward, presumably via actions partly mediated by MORs (Fig. 9A). The addition of maraviroc to Tat(−) mice did not influence morphine efficacy on these behavioral measures (Fig. 9B) consistent with *in vitro* reports wherein MOR-CCR5 bivalent ligands exert little influence when neuroinflammation is not present (Akgün et al., 2015; Portoghese et al., 2017). However, when a neuroinflammatory insult was first present [as modeled using Tat(+) mice], morphine efficacy was diminished on most measures; perhaps, due to endogenous MORs having already been cross-desensitized by activated CCR5 receptors (Fig. 9C). When maraviroc was introduced in the Tat-inflamed state, morphine efficacy was restored on most measures and even potentiated on the measure of reward. We speculate that the addition of maraviroc in this state may relieve cross-desensitization, restoring morphine signaling through its endogenous receptors (Fig. 9D). In support, a bivalent ligand (MOR agonist/CCR5 antagonist) exerted a 3100-fold increase in potency when administered to LPS-inflamed mice, relative to non-inflamed controls (Akgün et al., 2015; Portoghese et al., 2017). There was one behavioral exception to Tat's capacity to attenuate morphine efficacy and that was on the measure of reward prior to maraviroc. These divergent findings may involve the role of additional brain regions and opioid signaling beyond that involving CCR5 heterodimers (Shippenberg et al., 2009).

It must be noted that the timing of morphine and Tat-dependent interactions at CCR5 is likely to be critical in determining the nature of the outcome (see Fig. 9 in Berman et al., 2006; Song et al., 2011). The relative increases in cytokines seen with acute morphine and/or Tat exposure (for example, as in CCL3, CCL4, CCL5, CXCL1, IL-1α, IL-9, and IL-10) were often smaller effects that may either be less biologically significant or may display peak activity earlier than the time examined. These effects were largely absent after sustained exposure suggesting that the CNS adapts to sustained opiate or HIV-1 Tat insults via mechanisms involving drug tolerance (Eisenstein et al., 2006) or innate immune tolerance (Cavaillon & Adib-Conquy, 2006; Biswas & Lopez-Collazo, 2009), respectively. Future investigations should additionally assess the presence of endotoxemia which may contribute to these effects (Eisenstein et al., 2006), but may be masked by downregulation of the innate response.

The present work raises several clinical considerations. Seropositive patients within the first 100 d of HIV infection demonstrate positive correlations between circulating CCL2, CCL11, GM-CSF, IL-1α, IL-6, and lateral ventricular volume as well as IL-5, IL-10, and mean diffusivity within the caudate (Ragin et al., 2015). Herein, maraviroc exerted little influence over behavioral measures on its own, but attenuated the capacity for HIV-1 Tat to dampen morphine potency. Given that up to 40% of HIV-afflicted individuals experience distal polyneuropathies, headache, and additional chronic pain states (Keswani et al., 2002; Mirsattari et al., 1999) necessitating opioid medications for relief among a sizeable percentage (~23% of a 1400+ patient sample infected with HIV; Merlin et al., 2016), maraviroc may have potential benefits to improve opioid-based therapies. However, with chronic opioid use, the beneficial effects of maraviroc are less clear given that maraviroc was also associated with an increase in withdrawal symptomology and increased morphine reward. In addition, the neurotoxic actions of additional HIV proteins in the patient population, such as the HIV coat protein (gp120), may exert a divergent profile compared to that observed when HIV-1 Tat is expressed alone (Campbell et al., 2015; Maung et al., 2012; Mocchetti et al., 2013). The toxic effects of R5-tropic gp120 can be attenuated by morphine-mediated CCL5 upregulation, perhaps influenced by out-competition for CCR5 (Avdoshina et al., 2010). As such, future investigations may wish to target MOR-CCR5 bivalent strategies within the context of opioid dependency across a range of neuroAIDS models.

5. Conclusions

Several investigations using murine and human cell cultures have demonstrated neurotoxic interactions between the HIV-1 Tat, MORs, and CCR5. The present findings extend these data by investigating functional consequences of such interactions on morphine-mediated antinociception, tolerance, and reward in a murine model. Maraviroc blocked Tat's actions to attenuate the antinociceptive potency of acute morphine in non-tolerant mice. Intriguingly, maraviroc also potentiated the Tat-induced increase of morphine-CPP, even while it reduced the levels of many inflammatory chemokines and cytokines in the striatum including β-chemokines. Thus, while maraviroc is widely appreciated for its role in blocking HIV entry, it may be considered for additional therapeutic roles related to pain control in HIV-infected patients; albeit, caution may be warranted for individuals that are opioid-dependent or at risk for such abuse.

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Highlights

- HIV Tat decreases morphine potency and CCR5 antagonism attenuates Tat effects
- Tat decreases morphine withdrawal and CCR5 antagonism attenuates Tat effects
- Tat exposure and CCR5 antagonism attenuate the psychomotor response to morphine
- CCR5 antagonism potentiated Tat-promoted morphine conditioned place preference
- 24h post drug, Tat elevates central cytokines; CCR5 blockade attenuates Tat effects

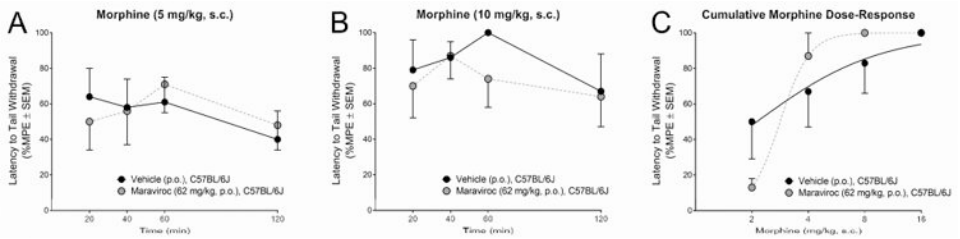

Figure 1. Maraviroc did not significantly influence the anti-nociceptive response to morphine in C57BL/6J mice

Latency to tail withdrawal (% MPE ± SEM) among C57BL/6J mice (n = 5 / group) that were administered vehicle (p.o.; black circles) or maraviroc (62 mg/kg, p.o.; blue circles) prior to (A) morphine (5 mg/kg, s.c.), (B) morphine (10 mg/kg, s.c.), or (C) a cumulative morphine dosing regimen (2 – 16 mg/kg, s.c.).

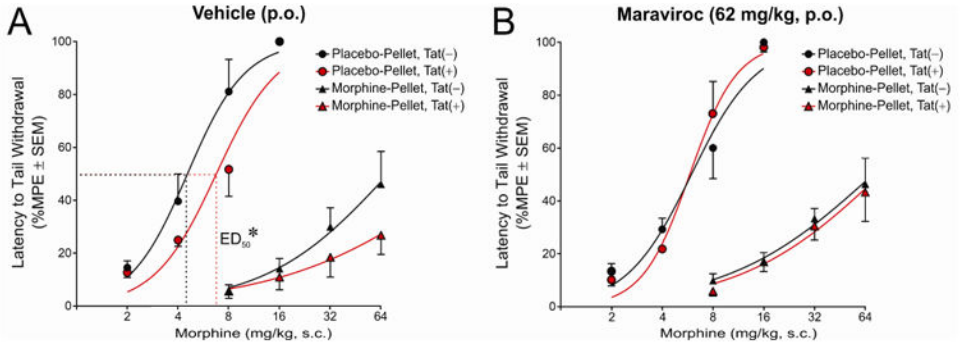

Figure 2. HIV-1 Tat significantly shifted the ED_{50} for morphine to the right in non-tolerant mice and maraviroc pretreatment obviated this effect

Latency to tail withdrawal (% MPE ± SEM) among Tat(−) or Tat(+) mice (n = 6 - 7 / group) that were morphine-naïve (placebo-pelleted; circles) or morphine-tolerant (morphine-pelleted; triangles). Mice [Tat(−) in black, Tat(+) in red] were administered (A) daily vehicle (p.o.) or (B) daily maraviroc (62 mg/kg, p.o.) following pelleting and were assessed for tail withdrawal reflex in response to a cumulative morphine regimen (2 - 64 mg/kg, s.c.). * indicates a significant shift in ED_{50}, $p < 0.05$.

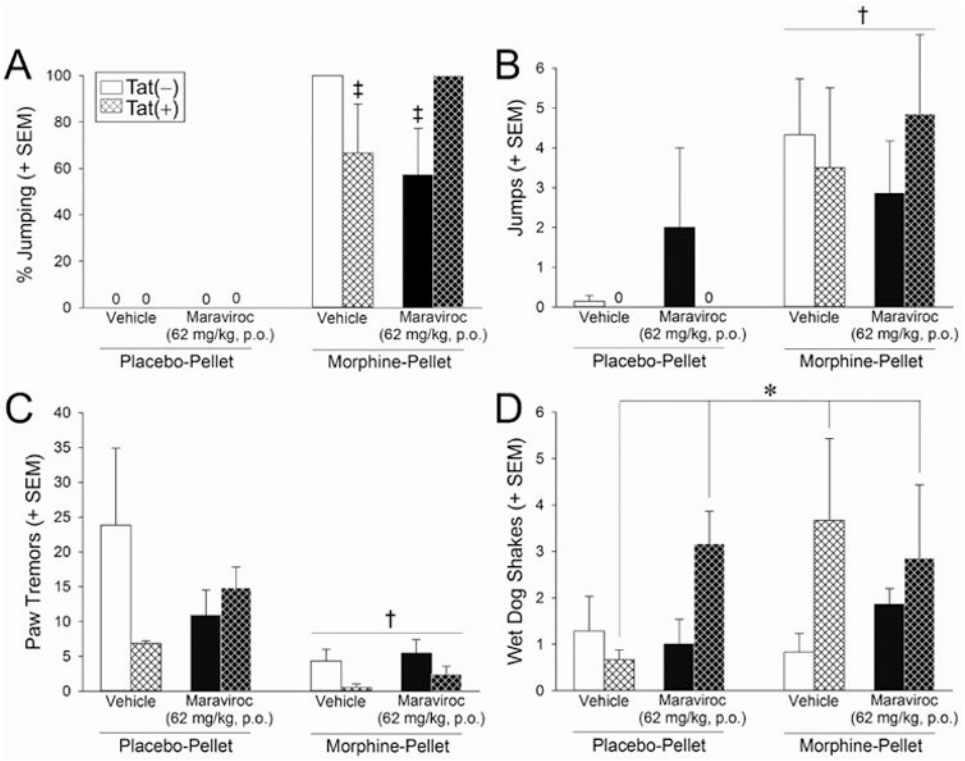

Figure 3. HIV-1 Tat significantly attenuated withdrawal-precipitated jumping and maraviroc pretreatment reversed this effect; Tat and morphine tolerance influenced additional withdrawal symptomology

Measures of naloxone-precipitated withdrawal among Tat(−) or Tat(+) mice [n = 6 - 7 / group; Tat(−) in open bars, Tat(+) in hatched bars] that were morphine-naïve (placebo-pelleted; left bars in each panel) or morphine-tolerant (morphine-pelleted; right bars in each panel), administered daily vehicle (p.o.; white bars) or maraviroc (62 mg/kg, p.o.; black bars), and assessed for warm water tail withdrawal. (A) The proportion of mice jumping from an elevated platform over 10 min, as well as the concomitant frequency of (B) spontaneous jumping, (C) paw tremor, and (D) wet dog shakes over 5 min in an observation box, were recorded. * main effect of Tat-exposure [greater wet-dog shakes among Tat(+) mice vs. Tat(−) mice]. † main effect of prior morphine tolerance (greater frequency of spontaneous jumps and fewer simultaneous paw tremors among morphine-tolerant vs. non-tolerant mice). ‡ interaction for indicated groups to differ from morphine-tolerant Tat(−) mice or morphine-tolerant, maraviroc-treated Tat(+) mice, $p < 0.05$.

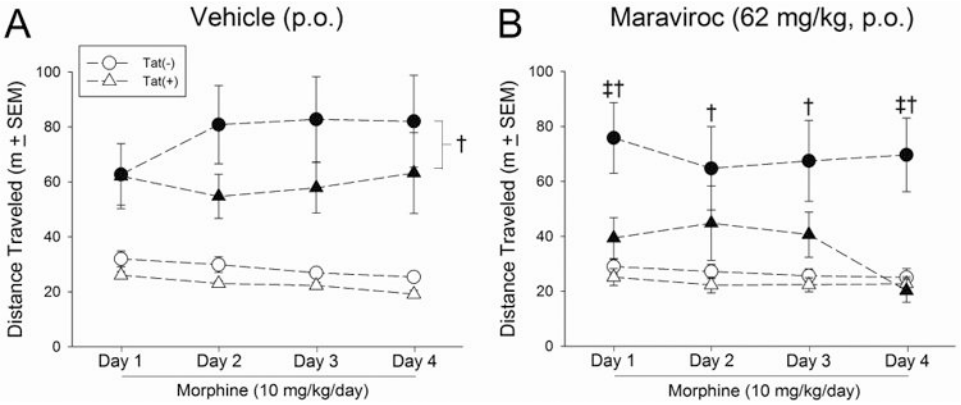

Figure 4. HIV-1 Tat and maraviroc significantly attenuate morphine-mediated psychomotor behavior

Locomotor behavior in response to saline (i.p. for 4 days; open circles) morphine (10 mg/kg, i.p. for 4 days; closed circles) was assessed during conditioning cycles among Tat(−) or Tat(+) mice that were pretreated with (A) vehicle (p.o.) or (B) maraviroc (62 mg/kg, p.o.; n = 11 - 12 / group). † main effect of morphine (greater locomotion following morphine vs. saline treatment). ‡ interaction for indicated Tat(−) groups differ from their respective Tat(+) counterparts, $p < 0.05$.

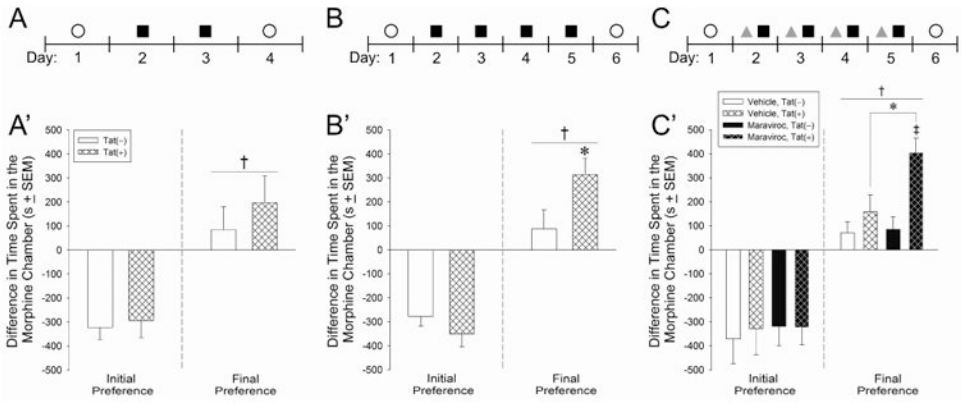

Figure 5. HIV-1 Tat potentiates morphine-conditioned place preference 24 h post morphine and maraviroc pretreatment exacerbates this effect

Tat(−) or Tat(+) mice (n = 11 - 14 / group) were assessed for their chamber preference (open circles) in a biased conditioned place preference (CPP) test consisting of either (A) two cycles of saline (i.p.)/morphine (10 mg/kg, i.p.) conditioning (closed squares), (B) four cycles of saline/morphine conditioning (closed squares), or (C) four cycles of vehicle (p.o.) or maraviroc pretreatment (gray triangles) followed by saline/morphine conditioning (closed squares). Initial preferences for the morphine-paired chamber (left of dashed line) are depicted followed by final CPP (right of dashed line) after (A′) two conditioning cycles, (B′) four conditioning cycles, or (C&prime) vehicle/maraviroc pretreatment prior to four conditioning cycles. Tat(−) mice are depicted in open bars, Tat(+) mice are depicted in hatched bars, vehicle (p.o.)-pretreated mice are depicted with white bars, and maraviroc (62 mg/kg, p.o.)-pretreated mice are depicted with black bars. † main effect for the final chamber preference to differ from the initial preference. * interaction for Tat(+) mice to differ from Tat(−) mice. ‡ interaction for maraviroc-pretreated Tat(+) mice to differ from all other groups, $p < 0.05$.

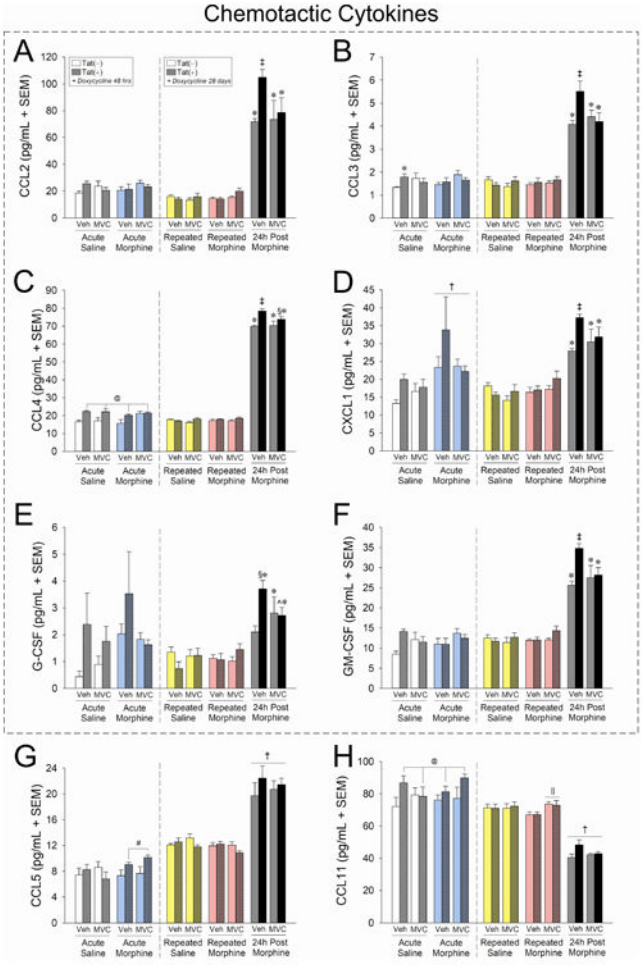

Figure 6. Chemokine expression in caudate/putamen is largely upregulated 24 h following repeated morphine exposure; HIV-1 Tat potentiated this effect on some analytes (outlined) which was ameliorated by maraviroc pretreatment

Chemotactic cytokine protein expression (pg/mL ± SEM) in dorsal striatum (caudate/putamen) of vehicle- (p.o.) or maraviroc- (62 mg/kg, p.o.) exposed mice [n = 4 – 5 / group; Tat(−) in open bars, Tat(+) in stippled bars] that had Tat expressed for 48 h or 28 days (via doxycycline). Mice were administered saline (i.p.; white bars) or morphine (10 mg/kg, i.p., QD) acutely (cyan bars), repeatedly (4 consecutive days; repeated saline in yellow bars and repeated morphine in magenta bars), or were 24 h post repeated morphine treatment (gray bars). Cytokines within the dashed box demonstrated Tat-potentiation that was ameliorated by maraviroc. @ main effect of Tat [Tat(+) mice have greater expression than Tat(−) mice, irrespective of morphine or maraviroc condition]. † main effect of morphine (indicated morphine group has greater expression than the respective saline group, irrespective of Tat or maraviroc condition). ‖ maraviroc-morphine interaction (indicated maraviroc-treated group differs from vehicle-treated control within the same morphine condition). Ϛ 3-way interaction for indicated groups to differ from vehicle-treated, saline-administered, Tat(−) controls. ‡ 3-way interaction for indicated group to differ from all other groups. § 3-way interaction for indicated groups to differ from respective vehicle-treated, Tat(−) control

within the 24 h post morphine group. ^ 3-way interaction for indicated maraviroc-treated Tat(+) group be reduced compared to respective vehicle-treated Tat(+) group, $p < 0.05$.

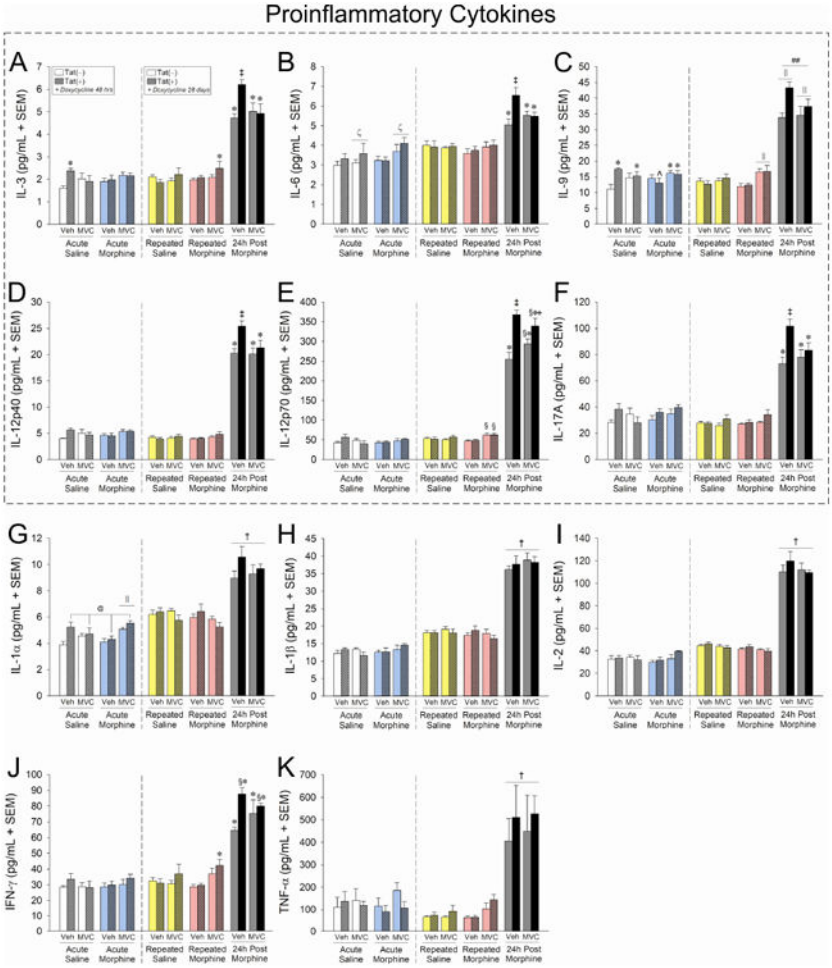

Figure 7. Proinflammatory cytokine expression in caudate/putamen is partly upregulated 24 h following repeated morphine exposure; HIV-1 Tat potentiated this effect on some analytes (outlined) which could be ameliorated by maraviroc pretreatment

Proinflammatory cytokine protein expression (pg/mL ± SEM) in dorsal striatum (caudate/putamen) of vehicle- (p.o.) or maraviroc- (62 mg/kg, p.o.) exposed mice [n = 4 - 5 / group; Tat(−) in open bars, Tat(+) in stippled bars] that had Tat expressed for 48 h or 28 days (via doxycycline). Mice were administered saline (i.p.; white bars) or morphine (10 mg/kg, i.p., QD) acutely (cyan bars), repeatedly (4 consecutive days; repeated saline in yellow bars and repeated morphine in magenta bars), or were 24 h post repeated morphine treatment (gray bars). Cytokines within the dashed box demonstrated Tat-potentiation that was ameliorated by maraviroc. @ main effect of Tat [Tat(+) mice have greater expression than Tat(−) mice, irrespective of morphine or maraviroc condition]. † main effect of morphine (indicated morphine group has greater expression than the respective saline group, irrespective of Tat or maraviroc condition). ζ main effect of maraviroc (maraviroc-treated groups have significantly greater expression that vehicle-treated groups, irrespective of Tat or morphine condition). ## Tat-morphine interaction wherein Tat(−) and Tat(+) mice in the indicated morphine group are greater than those in other morphine groups and differ from each other (irrespective of maraviroc condition). ‖ maraviroc-morphine interaction wherein maraviroc-pretreatment differs from vehicle-pretreatment within the indicated morphine groups

(irrespective of Tat condition). * 3-way interaction for indicated groups to differ from respective vehicle-treated, saline-administered, Tat(−) controls. ‡ 3-way interaction for indicated group to differ from all other groups. § 3-way interaction for indicated groups to differ from respective vehicle-treated, Tat(−) control within the 24 h post morphine group. ˆ 3-way interaction for indicated group to differ from respective vehicle-treated, acute-saline-administered, Tat(+) mice. ˆ 3-way interaction for indicated group to be greater than their maraviroc-treated, Tat(−) counterparts within the 24 h post morphine group, $p < 0.05$.

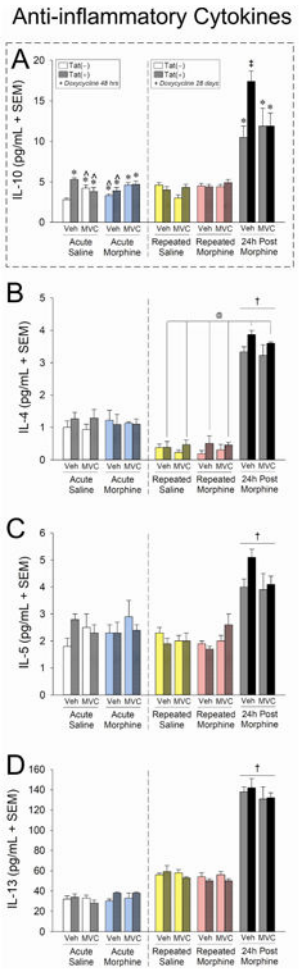

Figure 8. Anti-inflammatory cytokine expression in caudate/putamen is upregulated 24 h following repeated morphine exposure; HIV-1 Tat potentiated this effect on IL-10 and maraviroc pretreatment ameliorated this effect

Antiinflammatory cytokine protein expression (pg/mL ± SEM) in dorsal striatum (caudate/putamen) of vehicle- (p.o.) or maraviroc- (62 mg/kg, p.o.) exposed mice [n = 4 - 5 / group; Tat(−) in open bars, Tat(+) in stippled bars] that had Tat expressed for 48 h or 28 days (via doxycycline). Mice were administered saline (i.p.; white bars) or morphine (10 mg/kg, i.p., QD) acutely (cyan bars), repeatedly (4 consecutive days; repeated saline in yellow bars and repeated morphine in magenta bars), or were 24 h post repeated morphine treatment (gray bars). The dashed box indicates IL-10 as the only anti-inflammatory cytokine assessed that demonstrated Tat-potentiation with maraviroc amelioration. @ main effect of Tat [Tat(+) mice have greater expression than Tat(−) mice, irrespective of morphine or maraviroc condition]. † main effect of morphine (indicated morphine group has greater expression than the respective saline group, irrespective of Tat or maraviroc condition). * 3-way interaction for indicated groups to differ from vehicle-treated, saline-administered, Tat(−) controls. ‡ 3-way interaction for indicated group to differ from all other groups. § 3-way interaction for indicated groups to differ from respective vehicle-treated, Tat(−) control within the 24 h post morphine group. ˆ 3-way interaction for indicated group be reduced compared to respective vehicle-treated Tat(+) group, $p < 0.05$.

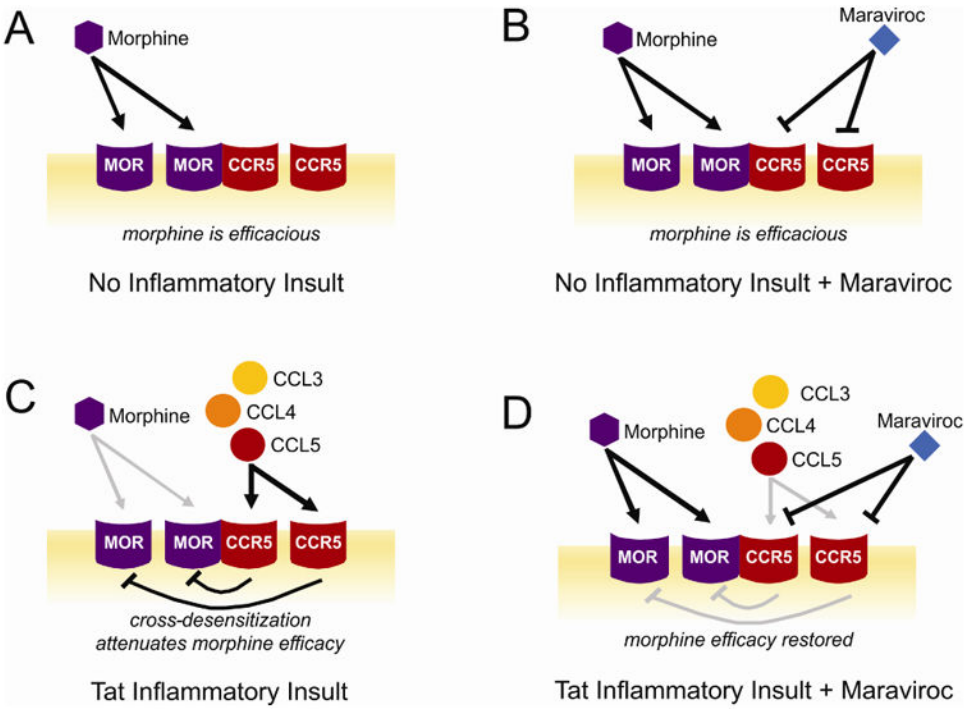

Figure 9. Proposed mechanism(s) of morphine/maraviroc interaction with or without HIV-1 Tat-induced neuroinflammation

(A) In the absence of Tat-mediated neuroinflammation [as modeled using (Tat-), control mice], morphine is behaviorally efficacious, partly via actions at mu opioid receptor (MOR) oligomers or MOR-CCR5 heteromers. MOR signaling may cross-desensitize CCR5 oligomers in this state (not depicted). (B) When no prior neuroinflammatory stimulus is present, the addition of maraviroc exerts little influence on the behavioral efficacy of morphine [as modeled using (Tat-), maraviroc-treated mice]. (C) When Tat-induced neuroinflammation is present [as modeled using Tat(+) mice], there are enhanced levels of β-chemokines that bind CCR5 and may cross-desensitize MORs. When morphine is administered in this state, its behavioral efficacy is attenuated. (D) The addition of maraviroc when neuroinflammation is present may block the ability of CCR5 to cross-desensitize MORs, thereby restoring morphine efficacy [as modeled using maraviroc-treated, Tat(+) mice].